

Genetic Polymorphism of GSTM1 as risk factor for Oral Potentially Malignant Disorder

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Abstract :

Background: Biotransformation plays a crucial role in carcinogen activity. Genetic polymorphisms in xenobiotic metabolising enzymes crucial to carcinogen and drug metabolism and lead to variations in their activity. These enzymes increase the risk of cancer by an altered action on environmental carcinogens. Tobacco abuse is a well-known risk factor for potentially malignant disorders. Factors that influence tobacco-exposed individuals developing a malignancy may include the combination of total tobacco exposure and genetic susceptibility.

The objective of this study was to analyse the significance of genetic polymorphisms in GSTM1 (carcinogen metabolizing enzymes) genes in patients with Oral Submucous Fibrosis (OSMF). Method: The study subjects included 50 patients. 25 were diagnosed with OSMF, 5 with malignant transformation in OSMF and 20 age and sex matched healthy controls. Genotypes of GSTM1 were determined by PCR-RFLP (Polymerase Chain reaction- Restricted Frequency Length Polymorphism). Results: 10%, 40% and 60% GSTM1 null genotype was observed in normal subjects, OSMF patients and OSMF patients with malignancy respectively. Conclusion: Polymorphism in GSTM1 null genotype may confer an increased risk for developing Oral Submucous Fibrosis.

Keywords : Genetic Polymorphism, GSTM1, Oral Submucous Fibrosis

Date of Submission: 18 -08-2017

Date of acceptance: 01-09-2017

I. Introduction

Genetic polymorphisms are prevalent and play a major role in human diseases especially oral precancers and cancers. Recently, the relationship of genetic polymorphisms and the risk of cancers have been researched widely. It is commonly considered that genetic polymorphisms and environmental factors including cigarette smoking, alcohol consumption, and betel quid chewing are of particular importance in the etiology of oral cancer^[1,2]. The environment-gene interaction in carcinogenesis is well reflected by Phase 1 and Phase 2 enzymes that are involved in the metabolism of carcinogens. Glutathione S transferases (GSTs) are a group of Phase 2 enzymes that are primarily involved in detoxifying carcinogenic metabolites^[3]. Numerous polymorphisms occur in the genes encoding GSTs. Among them, the null genotype of GSTM1 has a decreased capability in detoxifying some carcinogens present in tobacco. It exhibits a deletion polymorphism, which in case of homozygosity (GSTM1 null) leads to the absence of phenotypic enzyme activity. The GSTM1 (null) genotype has been found to be significantly associated with an increased risk of oral squamous cell carcinoma^[4]. Hence, the study was taken up with the aim of determining whether the presence of genetic polymorphism of GSTM1 genes predisposes the patients to Oral Submucous Fibrosis and subsequent malignant transformation.

II. Methodology

The study was conducted in the Department of Oral Medicine and Radiology in collaboration with Division of Cancer Research, Regional Cancer Center, Thiruvananthapuram for a period two years. The study was approved from the Institutional Ethics Committee. Patients who reported to the outpatient clinic were screened for features suggestive of Oral Submucous Fibrosis and their clinical data documented. Fifty Patients selected for the study were grouped into three namely, Normal, OSF and OSF with malignancy. Genomic DNA was checked using 0.8% agarose gel electrophoresis. The genetic polymorphisms of GSTM1 were analyzed by using PCR and RFLP by using specific primers. Beta globin was used as internal control and amplified in all the samples. Bands for GSTM1 genes were observed using gel doc system (**Fig 1**). Statistical analysis was done using SPSS. The various parameters were compared using chi-square tests. The association of observed values and clinical parameters was assessed using one-way analysis of variance (ANOVA).

III. Results

The age of the study subjects ranged from 25 – 75 years with a slight female predominance. In the normal group, only two patients had habits. All patients diagnosed with OSF and OSF with malignancy had the habit of betel quid chewing and also exhibited a higher frequency and longer duration of betel quid chewing; many of whom were chewers of commercial preparations of tobacco. 10% of the OSF patients included in the study were also diagnosed to have a leukoplakic patch on tongue. In this study, GSTM1 polymorphism was observed in normal subjects, OSF patients and OSF patients with malignancy to the extent of 10%, 44% and 60% respectively (**Fig 2**). By using chi square analysis it was noted that genetic polymorphism was higher in subjects diagnosed with OSF when compared to normal groups and the difference was statistically significant (p value - 0.013). Among subjects of both, OSF and OSF with malignant transformation groups, a greater percentage of females showed genetic polymorphism. Significantly, it was also observed that all of the subjects with GSTM1 polymorphism were habitual chewers of tobacco preparation. In the 18 normal subjects with deleterious oral habits, only two were polymorphic. Among the 30 OSF subjects including those with malignancy, it was observed that three had oral leukoplakia. Among the three, polymorphism was observed in two patients and both of them were only GSTM1 polymorphic. The twenty five OSF patients were divided into early, moderate and advanced grades according to histopathologic grading criteria of Sirsat and Pindborg^[5]. The study noted an increased expression of polymorphism in moderate cases. Among them eleven showed GSTM1 deletion alone.

IV. Discussion

Oral cancer has become a major health problem characterized by high incidence, poor survival rate, and severe functional and cosmetic defects accompanying the treatment^[6]. Moreover, it has been demonstrated that genetic and environmental factors could affect individual susceptibility to oral cancer. Therefore, it is significant to investigate the association of GSTM1 polymorphisms to oral cancer. Oral Submucous Fibrosis is the one of the most common oral potentially malignant disorder. The role of gene - environment interaction in carcinogenesis has recently gained much interest. Several studies indicate that polymorphisms in gene encoding enzymes that are involved in the metabolism of carcinogens present in tobacco smoke, alcohol and other environmental factors may be linked to individual susceptibility to oral cancer^[7]. Carcinogen-metabolizing enzymes are expressed in oral mucosa suggesting that metabolism of carcinogen could occur at this site^[8]. Differences in enzyme activity in the oral cavity could represent one mechanism by which oral cancer and precancers occur.

Epidemiological studies have demonstrated a remarkable influence of various habits in the development of oral cancer. So far, several epidemiological studies have evaluated the association of GSTM1 polymorphisms with oral cancer susceptibility. Haitao *et al* done a meta analysis to evaluate the association between GSTM1 polymorphism and susceptibility to cancer^[9]. The results demonstrated that null genotype of GSTM1 polymorphisms might serve as risk factors for oral cancer.

In our present study occurrence of GSTM1 genotype null deletion was significantly higher in OSF patients when compared to normal (p value 0.013). However with malignant transformation, even though an increased frequency of polymorphism was noted, the result was not significant. Sato *et al* and Hung *et al* are of the opinion that those who had null genotypes of GSTM1 had an increased oral cancer risk, whereas Park *et al* and Hahn *et al* reported that the null genotype of GSTM1 had no effect on the development of Squamous Cell Carcinoma^[10,11,12 &13]. The discrepancy between these studies may be due to several factors including difference in chewing habits and study subjects. Sreelekha *et al* suggested that polymorphism of GSTM1 null genotype in Indian subjects may confer an increased risk of oral cancer^[14]. Extensive review of available literature has not revealed any study on the relative risk of OSF in patients with genetic polymorphism of GSTM1. Durate *et al* has reported that GSTM1 polymorphism increases the risk of oral leukoplakia^[15]. Thus, oral leukoplakia, the most common precancerous lesions, has conclusively shown a positive association with GSTM1 null genotype. Among the 30 OSF subjects including those with malignancy in our study, it was observed that three had oral leukoplakia. Among the three, polymorphism was observed in two patients and both of them were GSTM1 polymorphic.

Genetic factors along with carcinogenic potential of the quid may increase risk of developing oral cancers and precancers. It was observed in the present study that of the 29 habitués with genetic polymorphism, 24 persons had developed OSF of whom four had malignancy as well. It is interesting to note that 82.7 % of persons with genetic polymorphism exposed to carcinogens had developed OSF. In the normal group, 18 had habits, in which two showed GSTM1 null deletion. Of the 18 normal habitué patients, 72.2% did not show genetic polymorphism (**Table 1**). This result implies that there are gene - gene and gene - environment interactions in the development of oral cancer. The risk of developing OSF among those polymorphic subjects in the normal group who had habits can be evaluated only after long term follow up studies. Since many carcinogens require metabolic activation before binding to DNA, individuals with an elevated metabolic capacity to activate specific carcinogens may be at increased risk of cancer. Null genotype of GSTM1 has

decreased capability in detoxifying some carcinogens. As a result of polymorphisms, the amount of carcinogens present in the body increases and this leads to cancers and precancers. In conclusion, our results suggest that polymorphism of GSTM1 null genotype increased risk of developing Oral Submucous Fibrosis. Accumulation of such information on individual susceptibility to certain type of cancers will be valuable for identifying high-risk individuals and to prevent possible development of cancer, both by reducing the intake of carcinogenic substances and introducing chemo-preventive measures.

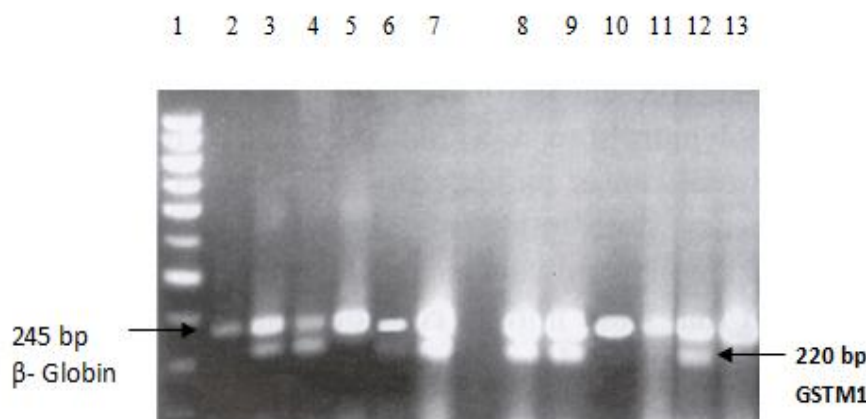
Acknowledgements

We would like to thank all the study participants for having taken part in the study. We express our gratitude to Mrs Sreelekha, Research Scientist and other staffs and students in the division of cancer research, RCC, Trivandrum, for their support and contribution in conducting this study.

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Figure 1



GSTM1 deletion in oral submucous fibrosis.
Lane 1 – 100 bp DNA marker
Lanes 2,5,10,11 & 13 show GSTM1 deletion

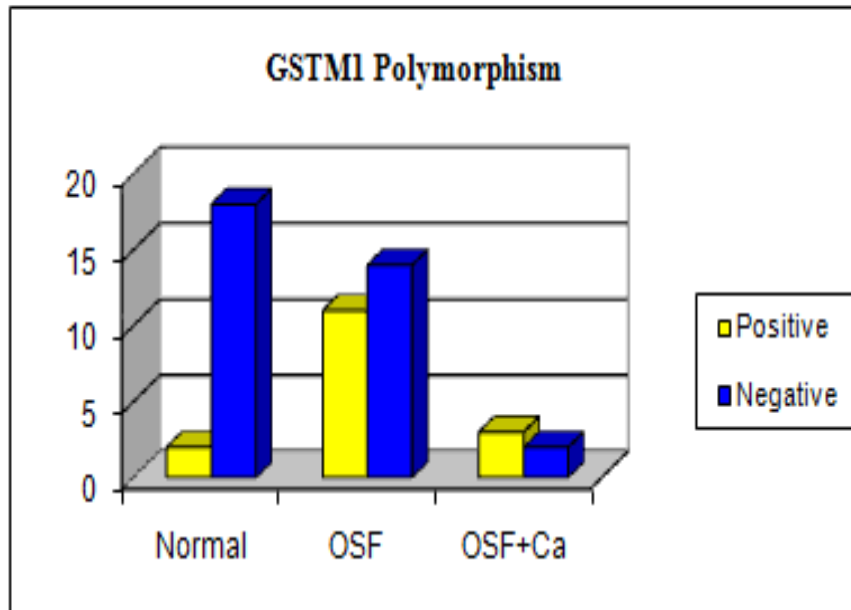


Table 1 Correlation of habit with GSTM1 polymorphism

Habit	Normal	OSF	OSF+Ca
Chew Only	0	8	2
Chew + Alcohol	1	3	1
Chew+Smoke+Alcohol	1	0	0
Total	2	11	3

*Sunu Ramachandran. "Genetic Polymorphism of GSTM1 as risk factor for Oral Potentially Malignant Disorder." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.8 (2017): 47-50